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Fast gradient screening of pharmaceuticals with 5 cm long, narrow bore reversed-phase columns packed with sub-3 μm core–shell and sub-2 μm totally porous particles

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ABSTRACT

The performance of 5 cm long narrow-bore columns packed with 2.6–2.7 μm core–shell particles and a column packed with 1.7 μm totally porous particles was compared in very fast gradient separations of polar neutral active pharmaceutical compounds. Peak capacities as a function of flow-rate and gradient time were measured. Peak capacities around 160–170 could be achieved within 25 min with these 5 cm long columns. The highest peak capacity was obtained with the Kinetex column however it was found that as the flow-rate increases, the peak capacity of the new Poroshell-120 column is getting closer to that obtained with the Kinetex column. Considering the column permeability, peak capacity per unit time and per unit pressure was also calculated. In this comparison the advantage of sub-3 μm core–shell particles is more significant compared to sub-2 μm totally porous particles. Moreover it was found that the very similar sized (d_p = 2.7 μm) and structured (ρ = 0.63) new Poroshell-120 and the earlier introduced Ascentis Express particles showed different efficiency. Results obtained showed that the 5 cm long narrow bore columns packed with sub-3 μm core–shell particles offer the chance of very fast and efficient gradient separations, thus these columns can be applied for fast screening measurements of routine pharmaceutical analysis such as cleaning validation.

1. Introduction

High separation efficiency and faster analysis have always been of great interest in liquid chromatography. A new period has been started with using sub-2 μm fully porous particles, silica monolithic rods and shell particles [1–4].

On sub-2 μm particles, due to the narrow peaks, sensitivity and separation are improved at the cost of pressure. Ultra-high pressure pump systems (UHPLC or VHPLC) have been used to overcome the high pressure drop generated by small particles of packing materials [1,2]. It was demonstrated that the analysis time could be reduced to a few minute interval without the loss of resolution and sensitivity using very fine porous particles [3,4]. The commercialization of UHPLC or VHPLC systems has accelerated applications of fast liquid chromatography in pharmaceutical analysis [5–10].

Temperature in liquid chromatography also offers a possibility to cut down the analysis time. Elevated temperature reduces the viscosity of mobile phase and hence increases the mass transfer. The separation time can be shortened significantly without loss of resolution through column and mobile phase heating [11–14]. However, this strategy suffers of limitations such as the small number of stable packing materials at temperatures higher than 80 °C as well as the potential degradation of thermolabile analytes and the need to have a constant temperature along the chromatographic system. Therefore, until now, the pharmaceutical industry has not considered this approach routinely [15].

Monolithic columns were introduced for their potential use at high mobile phase velocities due to decreased mass transfer effects over conventional fully porous particles [16,17]. The monolith approach, originally initiated by the work of Hjertén et al. [18], Svec et al. [19], Horvath and co-workers [20], Tanaka and co-workers [21], which already lead to a number of well performing, commercially available polymeric and silica monolith columns [22,23]. Due to the low phase and surface area per column ratio, retention on monolithic columns is generally lower compared to packed columns.

The development of core–shell or "superficially porous" silica particles was considered as a breakthrough in column technology aimed at reducing analysis times while maintaining column efficiencies and requiring relatively low back pressures [24,25]. A major benefit of the core–shell particles is the small diffusion path (0.23–0.50 μm) compared to fully porous particles (e.g.,

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1.7–1.9 μm). In theory, decreasing the thickness of the porous layer of porous material should cause a decrease of the C term in the van Deemter plot, because the length along which molecules should diffuse decreases [26]. Besides their structure, made of a shell around a solid core particle, the interesting feature of these superficially porous particles is their extremely narrow particle size distribution (PSD). Different authors report that this narrow PSD is the key for their success at separating small molecules [27–29]. Now core–shell packing materials are commercially available in different particle diameters (1.7 μm, 2.6 μm, 2.7 μm and 5 μm) with different shell thickness (0.23 μm, 0.25 μm, 0.35 μm and 0.50 μm).

The aim of this study was to make a practical evaluation of the possibilities of commercially available columns packed with sub-3 μm core–shell particles in gradient elution mode. We measured and compared the peak capacities for a mixture of 14 polar neutral active pharmaceutical ingredients (API, low molecular weight steroid and non-steroid hormone compounds, MW: 270–430 g/mol) which are often separated in pharmaceutical routine analysis. Three core–shell packing of similar size (Poroshell 120 SB-C18, Kinetex C-18 and Ascentis Express C-18) and a column packed with totally porous sub-2 μm particles (Waters Acquity BEH C18) were applied in order to compare the efficiency of the short, narrow bore reversed-phase columns in fast gradient elution. The peak capacities were measured with the same samples on each column, at constant chromatographic linear velocity and gradient steepness. We focused on fast gradient separations (gradient time was varied between 3 and 18 min) what can be useful for screening purposes for the measurements of hormones, steroids and their degradants, thus short (5 cm long) narrow bore (2.1 mm internal diameter) columns were used in this work. The fast screening gradient separations are very useful in pharmaceutical cleaning control analysis and in impurity testing of APIs.

2. Experimental

2.1. Chemicals, columns

Acetonitrile (gradient grade) was purchased from Merck (Darmstadt, Germany). For measurements water was prepared freshly using Milli-Q® equipment (Milli-Q gradient A10 by Millipore).

The test analytes were polar neutral small pharmaceutical active ingredient (API) compounds. Their molecular weights are in the range of 270–430 g/mol. All 14 test compounds (steroids, non-steroid hormones such as estrogens, progestins and anti-androgens and their main degradation products such as: gestodene (13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,15-dien-20-yn-3-one), levonorgestrel (13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one, (−)), estradiol (Estra-1,3,5(10)-triene-3,17β-diol), dienogest (17α-cyanomethyl-17β-hydroxyestra-4,9(10)-diene-3-one), finasteride (N-tert-butyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide), noretistherone-acetate (17-acetoxy-19-nor-17α-pregn-4-en-20-yn-3-one), bicalutamide (N-[4-cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenyl)sulfonyl)-2-hydroxy-2-methyl propanamide, (±)), tibolone (17-hydroxy-7α-methyl-19-nor-17α-pregn-5(10)-en-20-yn-3-one), ethinyl-estradiol (19-nor-17-pregn-1,3,5(10)-trien-20-yn-3,17-diol) and 5 degradation products (impurities) of ethinyl-estradiol (different hydroxy-, keto- and dehydro-ethinyl-estradiol degradants) were produced by Gedeon Richter Plc (Budapest, Hungary).

The Kinetex core–shell C18 column packed with 2.6 μm shell particles (50 mm × 2.1 mm) were obtained from GEN-Lab Ltd, Budapest. Ascentis Express C18 column (Supelco) with a particle size of 2.7 μm (50 mm × 2.1 mm) was purchased from Sigma–Aldrich Ltd., Budapest. Waters UPLC™ BEH C18 column with a particle size of 1.7 μm (50 mm × 2.1 mm) was purchased from Waters Ltd., Budapest. The recently introduced Poroshell 120 SB-C18 column with a particle size of 2.7 μm (50 mm × 2.1 mm) was a generous gift from Kromat Ltd., Budapest. All of the columns used in this study were new no other experiments were performed on them.

2.2. Equipment, software

All measurements were performed using a Waters Acquity system equipped with binary solvent delivery pump, an auto sampler and a photo diode array detector (Waters Ltd. Budapest, Hungary). The UPLC system had a 5 μl injection loop and a 500 nl flow cell (path length = 10 mm). A polyether ether ketone (PEEK) tube (15 cm × 0.1 mm) is located between the column outlet and the detector. The overall extra-column volume (V_{ext}) is 12 μl as measured from the injection seat of the auto-sampler to the detector cell at 1 ml/min. The measured dwell volume is 130 μl. Data acquisition with an 80 Hz data sampling rate and instrument control were performed by Empower 2 Software (Waters).

The non-linear curve fitting to peak capacity plots was performed using Statistica 9.0. Image J (freeware image-processing software program developed at the National Institutes of Health) was used to determine the particle size and the size distribution of column packing materials.

2.3. Apparatus and methodology

The mobile phase "A" was pure water while the mobile phase "B" was pure acetonitrile. The eluents were degassed by sonication for 5 min.

The stock solutions of each test solute were set in acetonitrile (1000 μg/ml). The stock solutions were sonicated for 5 min in ultrasonic bath then they were homogenized. After that 1 ml stock solutions of each solute were transferred into a 50 ml volumetric flask. A sample-solvent of acetonitrile/water 40/60 v/v was added to the flask to give the final volume. The concentration of the final pharmaceutical test mix was 20 μg/ml (for each solute).

Preliminary gradient runs were performed to find the final gradient experimental conditions. According to the preliminary results, a linear gradient from 40% B to 90% B provides sufficient retention on all four columns and baseline resolution at least for 8–9 compounds.

The volume fractions of acetonitrile at the beginning and at the end of the gradient were set constant at 40 and 90%, respectively ($\Delta\varphi = 0.50$). It was followed by column equilibration (at the end of each gradient run, the 90% B was held for 1 min then the initial gradient condition was reset and run for 2 min to equilibrate the system). Solvent strength was varied linearly with times ranging from 3 to 21 min (t_g = 3, 6, 9, 12, 15, 18 and 21 min) being employed. The efficiency of each column was investigated at six different chromatographic linear velocities (u_0 = 0.144, 0.193, 0.241, 0.289, 0.337 and 0.385 cm/s). The columns were thermo-stated at 30 °C. The injected volume was 0.5 μl (partial loop with needle overfill mode), and UV detection at 215 nm (80 Hz) was applied. Since all experimental parameters have been kept constant, these conditions can be used to effectively compare the efficiency of the 5 cm long 2.1 mm internal diameter core–shell columns in fast gradient separation. For each linear velocity, six different gradient times t_g were applied in order to allow a direct comparison of the peak capacity P_c between the four columns at constant gradient analysis time. A total of 6 × 7 × 4 = 168 chromatograms were recorded, corresponding to six different linear velocities, seven different gradient time and four different columns.

The extra-column variance of our UPLC system was measured around 6–7 μl^2 depending on the flow-rate [41,42]. In gradient